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Review Article

Sperm Interactions from Insemination to Fertilization

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Contents

The task of spermatozoa is to transport its DNA-load as efficiently and safely as possible from the male organism to the female. Before it reaches its destination, it has to pass almost through the entire female reproductive tract, a potentially hostile environment. During passage, it is confronted by a sophisticated system that provides sperm storage sides but also possibly facilitates selection. The present review attempts to summarize the current knowledge of sperm interactions during that journey. A better understanding of the highly complex processes taking place between insemination and fertilization will be necessary to improve the efficiency of conventional reproductive techniques as well as for enabling the development and establishment of new ones.

Introduction

During mating, billions of spermatozoa are released into the female genital tract making successful fertilization most likely. Sperm transport through the genital tract towards the oocyte is regulated and highly efficiently controlled by the different compartments of the female genital tract, placing filters and traps into the path of spermatozoa. Only a very few selected spermatozoa finally reach the site of fertilization (Suarez 2003). The precise knowledge of these selective mechanisms cannot be overestimated to optimize the use of semen from domestic farm animals when used for artificial insemination. However, the precise interactions of spermatozoa in the time-span between their release into the female reproductive tract (FRT) and their final encounter with the ovum are complex and so far only partly understood.

It is generally accepted that in domestic mammals for successful fertilization, an oviductal sperm reservoir has to be established, which consists of several thousands of spermatozoa. But to archive these seemingly moderate numbers using conventional AI techniques, the insemination dose has to contain several million, up to a billion spermatozoa, a fact which greatly limits the efficient use of ejaculates and the establishment of new techniques like the use of sexed spermatozoa.

While the interactions of spermatozoa with the oviducts are comparatively well studied, information about the fate of spermatozoas before they actually get there is relatively scarce. This is somewhat surprising considering that fertilization failure results mostly from a lack of competent spermatozoa arriving the site of fertilization, i.e. the oviducts (Hill et al. 1971; Hunter and Wilmut 1984). Even though it has been proven in several species that spermatozoa reach the oviducts within minutes after insemination these early arrivals do not seem to take part in fertilization (Overstreet and Cooper 1978; Overstreet and Tom 1982; Hawk 1983). There is a considerable time span between insemination and the establishment of a reservoir of competent spermatozoa at the oviducts, for instance approximately 8 h in cattle and sheep (Hunter et al. 1980; Hunter and Wilmut 1984) and 1–2 h in swine (Hunter 1981). Knowledge about sperm interactions during this time span might enable us to establish a sufficiently big sperm reservoir in the oviducts working with conventional easy-to-handle AI methods without using copious amounts of spermatozoa.

Possible partners for sperm interactions before the sperm–oocyte rendezvous are partially species dependent and include cervical, uterine and oviductal epithelial cells (OEC), and also a wide array of immune cells known to be present in the lumen of the female reproductive tract at the time of ovulation. Another set of factors to be considered is the fluids present in the reproductive tract at the time of the interaction such as seminal plasma and cervical/uterine/oviductal secretions, which include oestrogen-associated glycoproteins affecting fertilization (Killian 2003). Also semen extenders have been shown to have an impact on sperm interactions (Taylor et al. in press).

In the following, the current knowledge on this matter will be reviewed starting at the most caudal point of the FRT where spermatozoa are delivered in a species dependent manner.

The cervix

The place of semen deposition varies between species. Thus the cervix is of particular importance for sperm interactions, where the ejaculate is deposited in the vagina close to the posterior end of the cervix, in species such as rodents, cattle and sheep. However, despite their similarity concerning sperm deposition, considerable differences occur between these species in the further progress of the sperm population. While in rodents, more or less the entire inseminate including the seminal plasma is swept into the uterus within minutes (Bedford and Yanagimachi 1992; Carballada and Esponda 1997), the same process stretches over more than 24 h in cattle and sheep (Mitchell et al. 1985). In murine and other rodents, a portion of the ejaculate coagulates into a copulatory plug thus forming a seal to prevent excessive retrograde sperm loss or

to prevent the access of semen from other males (Matthews and Adler 1978; Carballada and Esponda 1992). In contrast, cows do not form such a tight plug and lose up to 60% of inseminated spermatozoa via backflow (Mitchell et al. 1985).

Especially in cattle and sheep, the cervix has been suggested to serve as a sperm reservoir (Mattner 1968; Hawk and Conley 1975). The long persistence of spermatozoa in this particular area (Mitchell et al. 1985) seems to support this hypothesis. It is presumed that spermatozoa remain within the many crypts and folds of the cervix (Hawk 1987). However, no exact mechanism has been described yet on how spermatozoa are detained there or on what stimulus they are to be released. So far, no direct interaction between cervical epithelial cells and spermatozoa has been reported.

Apart from storage, the cervical passage might also serve as a selective barrier for spermatozoa. Especially the viscous mucus that fills the cervical lumen is considered a formidable obstacle for immotile and morphologically abnormal spermatozoa (Barros et al. 1984; Pretorius et al. 1984; Ragni et al. 1985). In contrast, motile spermatozoa might even benefit from it, because their ability to orientate along the long axis of threads of bovine cervical mucus has been demonstrated (Tampion and Gibbons 1962). Furthermore, in studies on the functional cervix anatomy, mucosal folds were described forming longitudinal channels along the periphery of the cervix leading straight from the external os of the cervix into the uterus (Mullins and Saacke 1982, 1989). Histochemical examination of the cervical mucus showed that during the follicular phase, the canals contained less dense mucus than in the central part of the cervical canal, thus permitting motile spermatozoa to travel easier, while damaged spermatozoa and micro-organisms are trapped in the retrograde moving highly viscous central mucus. During predominantly progesterone controlled cycle phases, the entire mucus is less watery and is nearly impenetrable to spermatozoa (Croxatto 1996).

The uterus

Spermatozoa enter the uterus depending on species either after cervical passage or directly after being ejaculated together with varying amounts of seminal plasma. Thus inevitably, differences will occur in the ways the female organism deals with the spermatozoa. A comparatively well-researched species concerning sperm-uterine interaction is the pig. Porcine spermatozoa are deposited straight into the uterus regardless whether insemination takes place naturally or artificially. Thus the uterus represents not only the first line of defence against possible invading pathogens but also might act as a sperm reservoir and sperm selection side similar to the proposed tasks of the cervix in cattle and sheep.

There is evidence collected in several studies on pigs that seems to point towards a storage and possibly selection of spermatozoa within the uterus before they are allowed to proceed to the oviducts. It is for instance known that even though spermatozoa can be found in the oviducts as shortly as 5–15 min after insemination (First et al. 1968; Baker and Degen 1972), the overall population in the oviducts never exceeds several thousand. The uterus on the other hand harbours a pool of several million for up to 24 h (First et al. 1968; Pursel et al. 1978). Furthermore, it was proven that a circumvention of the uterus facilitated successful insemination with a fraction of the usual sperm dosage (Johnson 1991; Vazquez et al. 2005). Rather than a mucus-based barrier, this selection process might rely on direct interactions between spermatozoa and uterine epithelial cells (UEC). This is confirmed and specified by the results of microscopic examinations of the uterine epithelium and the epithelium of the utero-tubal junction after insemination, where porcine spermatozoa were observed bound to epithelial cells (Lovell and Getty 1968; Rodriguez-Martinez et al. 1990). In these studies, it remained unclear whether this connection was selective to a certain sperm subpopulation, how it was mediated and what its biological consequences were. Interestingly, it was described that the spermatozoa attached to the epithelial cells mostly showed normal sperm ultrastructure, while most free spermatozoa were noticed to have damaged plasma membranes (Rodriguez-Martinez et al. 1990). These findings are supported by recent results obtained in *ex vivo* trials where the binding to the UEC seemed to be restricted to a subpopulation of spermatozoa with intact outer membranes and an existing mitochondrial membrane potential, i.e. viable and motile spermatozoa (Taylor et al. 2008). A study performed on cows came to a similar conclusion after insemination directly into the uterus with live or heat-killed spermatozoa. The results showed that within 1 h, 96% of the heat-killed spermatozoa had been discharged into the vagina, while only 26% of the live spermatozoa had suffered the same fate (Suga and Higaki 1971). Thus, like in pigs, the viable spermatozoa seem to be retained within the uterus. In both cases, though also unattached, viable spermatozoa were noticed indicating that the binding may be transient or that only a special subpopulation of spermatozoa is able to attach themselves to the epithelium. However, no definite answers can be given what exactly makes up the molecular nature of this connection. It might be similar to the lectin-mediated connection between spermatozoa and OECs, which will be discussed in depth later. Hypothetically, the involvement of integrins is also a feasible option. Integrins have already been shown to play a role in nidation and placentation of several species (Sueoka et al. 1997; Reddy and Mangale 2003) and at least in the bovine uterus, their expression depends on the level of steroid hormones (Kimmins and MacLaren 1999). Further research will be necessary though to give definite answers.

It is an important finding that the bound spermatozoa can be considered viable to understand the biological relevance of the sperm-UEC binding process. In this respect, it appears to be similar to the binding of spermatozoa to oviductal cells in the utero-tubal junction and distal oviductal isthmus. Here also, only viable spermatozoa attached themselves to the epithelial cells and gained a prolonged lifespan from this attachment (Fazeli et al. 1999; Topfer-Petersen et al. 2002). Whether the latter applies also to interactions between spermatozoa and uterine cells remains to be proven.

However, evidence exist that at least in humans, contact to UECs significantly improves sperm motion parameters (Fusi et al. 1994; Guerin et al. 1997). Nevertheless, the fact that spermatozoa indeed benefit from the attachment to the UECs in the ways described, would only seem sensible if they were to be released again by the epithelial cells to move on towards the oviducts and the oocytes. It was observed in gilts that while the sperm population in the oviducts remained stable for over 24 h containing between 2000 and 16 000 spermatozoa, the population in the uterus decreased rapidly, but still consisting of roughly half a million after 24 h (Pursel et al. 1978; Kunavongkrit et al. 2003). Pursel et al. (1978) suggested that the reservoir in the oviducts is fed restrictively by a larger reservoir in the uterus. Present results confirm these observations (Taylor et al. 2008). A possible explanation for such proceedings might be the desire of the female organism to compensate for different time intervals between standing oestrus and ovulation. Hypothetically, of freshly ejaculated spermatozoa, those having a more advanced stage of fertilizing competence might connect directly with the oviducts and are perhaps not recognized by the selective mechanisms of the uterine horn, whereas viable spermatozoa being in a less mature stage when entering the uterus might attach themselves to the uterine wall. Thus, if ovulation occurs a considerable time after insemination, the viable spermatozoa from the uterine reservoir had time to mature and proceed to the oviducts to refill in the oviductal reservoir and replace the former spermatozoa, which have outlived their lifespan.

When using highly diluted semen in AI with less than 0.2 to 2% of the original sperm number' such uterine reservoir might not or insufficiently be built up and may shorten the availability of spermatozoa released from the utero-tubal depot. Sperm related differences to built up such secondary depot might be a reasonable explanation that the success of insemination with highly diluted ejaculates is very much donor specific (Den Daas et al. 1998). This could be critical if the life span of spermatozoa is significantly reduced as it is for example in sex sorted spermatozoa (Klinc et al. 2007).

Everything mentioned so far has been under the conception that the binding of spermatozoa in the uterus is part of a positive selection. However, the opposite is also a possible option. It was shown that the presence of seminal plasma leads to fewer viable spermatozoa binding to the epithelial cells (Taylor et al. 2008). As seminal plasma is generally looked upon as a protectant of spermatozoa, this might indicate that the binding to the uterine wall is actually of disadvantage for a spermatozoon, literally being hindered to ascend.

Concerning the biological consequences of interactions between spermatozoa and UECs, another aspect should also be considered namely what effect the attachment has on the epithelial cell. In general, seminal plasma is looked upon as the elicitor of post-insemination changes on the uterine tissue such as the redistribution of leucocytes (O'Leary et al. 2004) and the induction of ovulation (Waberski et al. 1995, 1997). However, Rozeboom et al. (1998, 1999) noticed in pigs an increase in the migration of neutrophilic granulocytes into the uterus after insemination, if the insemination dose included spermatozoa compared with extension media alone. Thus, there should be ways, how spermatozoa can make their presence known in the uterus. Among several options, one could be via communication with the UEC, which in response to the interaction starts to produce cytokines to alert the immune system. However, trials on mice could not detect such an effect on the cytokines when examined (Robertson et al. 1996). Nor did they in fact, in opposite to Rozeboom et al. (1998, 1999), detect an over-average rise in the neutrophil-influx into the uterus specifically because of spermatozoa. But the circumstance that the respective authors examined the uteri of different species at different times after insemination makes a comparison difficult. Contradictory to the above, a recent *in vivo* study seemed to indicate that at least in pigs, spermatozoa do indeed have regulating influence on epithelial cytokine expression, if somewhat different than expected. Compared with the controls, three of five tested cytokines were down-regulated to baseline-levels in the presence of spermatozoa (Taylor, Schuberth, Rath, unpublished observations).

Besides epithelial cells, spermatozoa are also confronted with leucocytes during their uterine passage. In pigs, around oestrus, polymorphonucleic neutrophilic granulocytes (PMN) congregate all the way through the uterine endometrium along the basal lamina of the surface epithelium (Bischof et al. 1994a; Kaeoket et al. 2002a,b). Insemination causes a considerable number of these neutrophils to proceed through the basal lamina into the surface epithelium and the uterine lumen (Rodriguez-Martinez et al. 1990; Bischof et al. 1994b; Kaeoket et al. 2003; Taylor et al. in press). Indeed, some of them appear to cross into the uterus even without such a challenge, forming together with migrated monocytes, a resident uterine leucocyte population (Rozeboom et al. 1998, 1999; Matthijs et al. 2003; Taylor et al. in press). Similar results have been reported in horses and mice (Kotilainen et al. 1994; Tremellen et al. 1998). In contrast, it was not possible to show similar effects in the bovine uterus, independent from the sperm pre-treatment and sperm quality (Wendt 2007). In rabbits, neutrophils have been reported to migrate into the vaginal lumen post-coitus (Phillips and Mahler 1977), a finding, which might also be true for other species depositing sperm in vagina or cervix. Thus, it seems that spermatozoa arriving at the female genital tract see themselves confronted with a growing population of neutrophils.

The biological relevance of this event is not yet quite clear. It has been suggested that accidentally appearing micro-organisms as well as neutrophils target and remove preferentially aged, dead or prematurely capacitated spermatozoa (Vogelpoel and Verhoef 1985; Matthijs et al. 2000, 2003; Eisenbach 2003). However, so far evidence for it remained inconclusive. Equally unresolved is the question what molecular structure PMN are supposed to recognize on the sperm surface. There are several ways for a neutrophil to recognize its target like opsonization with complement factors or antibodies, recognition of a specific structure on the surface of the target cell such as toll-like receptors or lectinophagocytosis (Ofek and Sharon 1988). Even

without any mediators like opsonins or specific surface structures, some particles will be phagocytosed (Beukers et al. 1980). According to *in vitro* studies in pigs, which were performed with washed spermatozoa and peripheral blood neutrophils, damaged and capacitated spermatozoa were only targeted by neutrophils in the presence of serum, i.e. opsonizing factors such as complement and antibodies, even though heat inactivated serum sufficed in the case of damaged spermatozoa (Matthijs et al. 2000). Both factors are likely to be present in the uterus, because complement production in the UECs has been proven in some species (Sundstrom et al. 1989; Balan et al. 2001; Li et al. 2002) and even though natural anti-sperm antibodies might not actually exist (Kalaydjiev et al. 2002), at least multiparous females might possess acquired antibodies. Interestingly, a certain part of the uncapacitated, viable and motile spermatozoa population was able to attach themselves to PMN without the aid of opsonizing factors (Matthijs et al. 2000; Taylor et al. 2008). Thus it seems that the interaction between PMN and viable spermatozoa is partly facilitated by a direct ligand–receptor connection. Lectins are unlikely candidates for facilitating sperm–neutrotrophil binding (Taylor et al. 2008). Integrins may play a role, even though their involvement has only been proven in humans and, furthermore, requires the presence of anti-sperm antibodies (D'Cruz and Haas 1995). Possibly, no specific surface molecules at all are involved. Simply opposing surface charges suffice for neutrophils to attach themselves to particles and to subsequently phagocytose them (Beukers et al. 1980). This would also explain why membrane-damaged spermatozoa do not bind to PMN unless marked by opsonins, as they cannot maintain an electric membrane potential.

As neutrophils are mainly viewed as classic phagocytic cells, their role in the uterus was somehow reduced to this function. However, phagocytosis was not always reported as the end-result of the connection (Taylor et al. 2008). Another possible option to be considered is the inducement of apoptosis, as it has been proven that neutrophils are able to do so (Wang et al. 2007). More spermatozoa could be killed in this way in a shorter time and thus stopped to proceed to the oviducts. The thus damaged spermatozoa might be subsequently removed via backflow, which indeed has been proven to be an efficient tool in removing damaged spermatozoa (Suga and Higaki 1971). This scenario would also avoid the development of a massive inflammation, which one would expect to issue if several million of neutrophils die en masse in the uterus after phagocytizing billions of spermatozoa. But whether the result of the attachment is phagocytosis or apoptosis, the consequences would be dire for the spermatozoa. However, a positive outcome is also conceivable. Perhaps the subpopulation of PMN-bound spermatozoa even profit from this situation by being marked in some way or receiving stimuli to aid their maturation and are subsequently released again. Against that option speaks the finding that seminal plasma inhibits the adherence of spermatozoa to neutrophils *in vitro* significantly (Gilbert and Fales 1996; Binks and Pockley 1999; Taylor et al. 2008). Finally, it should also be considered that, whatever the outcome for the spermatozoa, the attachment of a spermatozoon to a PMN might be of importance for the regulation of the uterine immune response by activating the neutrophils to produce immune-regulatory cytokines, which in turn could enhance or subdue further neutrophil migration into the uterus or cause other alterations in the distribution of leucocytes in the endometrium.

The oviduct

The oviduct is the best researched part of the female genital tract concerning sperm interactions and has been the sole subject of several excellent reviews (Bosch and Wright 2005; Rodriguez-Martinez 2007). It is divided into three compartments with distinct physiological functions. Its most anterior part, the infundibulum, is exclusively responsible to transport the ovum to the site of fertilization, the ampulla. Between ampulla and uterus lays the isthmus with the utero-tubal junction. The latter represents in domestic mammals a very well studied sperm reservoir (Hunter 1981, 1984; Suarez 1987; Thomas et al. 1994a,b). Because of the information on comparative abundance, it is easier to appreciate the complexity of the mechanisms serving to trap, store and release spermatozoa, which to a certain extent is most probably also applicable for other less well understood sperm reservoirs elsewhere in the female genital tract. The management of the sperm reservoir is the result of a finely orchestrated coordination of the patency of the oviductal lumen, mucus secretions, oviductal fluid secretions, temperature gradient and receptor–ligand interactions between spermatozoa and OECs. To gain entrance into the oviducts, spermatozoa have to pass through the utero-tubal junction that in itself already represents a formidable obstacle. The lumen is not only particularly narrow and twisted, but also additionally complicated by mucosal folds and dead end grooves (Suarez 1987; Wrobel et al. 1993; Yaniz et al. 2000). In the lamina propria of the wall, a vascular plexus is situated that, supported by a thick muscular layer, forms a physiological valve, which might aid further constriction of the lumen (Hook and Hafez 1968; Wrobel et al. 1993). Furthermore, a viscous mucus filling the tight lumen of the utero-tubal junction and the adjacent isthmus has been described in cattle (McDaniel et al. 1968; Suarez et al. 1997), pigs (Hunter 1995; Rodriguez-Martinez et al. 2001) and rabbits (Jansen and Bajpai 1982). Similar to the mucus in the cervix of cattle and sheep, it might prevent damaged sperm from passing while promoting the ascendance of healthy spermatozoa. It has also been suggested that the mucus aids sperm storage in the isthmic region by suppressing sperm flagellar movement similar to the mechanism in the cauda epididymis, where motility is also inhibited by highly viscous mucus (Overstreet et al. 1980). Interestingly, it takes more than intact membranes and a good motility for a sperm to pass trough the utero-tubal junction. Spermatozoa of mice, which are null mutants for genes responsible for the expression of certain sperm surface proteins, are not able to pass through the utero-tubal junction, even though neither their motility nor morphology is impaired (Krege et al. 1995; Ikawa et al. 1997; Cho et al. 1998). The findings indicate that

apparently a direct contact between spermatozoa and the epithelium of the utero-tubal junction is required to facilitate the passage.

Once through the utero-tubal junction, the spermatozoa have reached the side of the final sperm reservoir that is formed by spermatozoa binding directly to the OECs (Hunter 1981; Suarez 1987). The reversible process is mediated via carbohydrate residues on the luminal surface of the epithelial cell and corresponding lectins expressed by spermatozoa. The involved oligosaccharide moieties differ between species [Hamster: Sialic acid and fetuin (DeMott et al. 1995); Horse: Galactose (Dobrinski et al. 1996); Pig: Mannose (Topfer-Petersen et al. 2002); Cattle: Fucose (Lefebvre et al. 1997)].

It has been shown that the sugar-binding lectin on the surface of bull spermatozoa is a protein derived from seminal plasma (Ignotz et al. 2001; Gwathmey et al. 2003). The protein called PDC-109 (BSP-A1/A2) is a product of the seminal vesicles and thus does not bind to the sperm plasma membrane until after ejaculation. The hypothesis is supported by the observation that ability to associate with OECs is reduced in epididymal sperm (Petrunkina et al. 2001; Gwathmey et al. 2003). However, it remains to be found whether similar mechanisms exist in other species too.

The manner in which the storage, especially release of the epithelial-bound spermatozoa is managed has not yet been clearly understood. As binding of post-capacitated spermatozoa is infrequent, it has been concluded that capacitational changes involving sperm plasma membrane and cytoplasm are most likely the cause for the disengagement of the spermatozoa from the oviduct epithelium (Lefebvre and Suarez 1996; Fazeli et al. 1999; Revah et al. 2000). It has been speculated that a capacitation-induced shedding or modification of the binding proteins facilitates the release (Bosch and Wright 2005; Suarez and Pacey 2006). However, what exactly leads up to capacitation still needs to be determined. Multiple factors, of chemical as well as of physical nature, have been suggested that probably all play a role to a certain degree. Unsurprisingly, they all seem to stand under a certain degree of hormonal control. The chemical factors influencing the spermatozoa stored at the oviductal reservoir are found in the composition of the isthmic mucus and the oviductal fluid. As mentioned above, the mucus probably aids preovulatory sperm storage by subduing sperm motility (Overstreet et al. 1980) and perhaps also by retarding sperm transport (Suarez et al. 1997). However, rising oestrogen-levels lead to an enrichment of the mucus with bicarbonate, which promotes capacitation and thus might prepare the spermatozoa for release and subsequent fertilization (Rodriguez-Martinez et al. 2001). The glycosaminoglycan hyaluronan, which also reaches its maximum concentration during oestrus, has been shown to antagonize this process, possibly acting as a regulating opponent to bicarbonate (Suzuki et al. 2000, 2002; Rodriguez-Martinez et al. 2001).

The ion composition in the oviducts, which differs from serum levels particularly regarding the concentration of potassium and calcium, has also been implicated in modulating sperm storage. It has been shown that in cows, oviduct potassium levels are raised constantly above serum levels, while calcium peaks at oestrus, only to fall again rapidly, reaching serum levels on day 2 of the oestrous cycle (Hugentobler et al. 2007). The reason for the elevated calcium levels around oestrus lays most probably mainly in the role of the cation in the initiation of capacitation. The raised potassium concentration in the oviducts is as yet unexplained, but it has been shown that potassium inhibits motility and thus might aid sperm storage (Burkman et al. 1984).

Other chemical factors with a potential role in releasing spermatozoa from the oviduct epithelium are the glycosidases. Very recent studies have determined that porcine and bovine oviductal fluid display α-L-fucosidase, β-N-acetyl-glucosaminidase, β-D-galactosidase, α-D-mannosidase and β-N-acetyl-galactosaminidase activity at physiological pH with variations along the different phases of oestrous cycle (Carrasco et al. 2007; Romar et al. 2007), suggesting a hormonal regulation of such an activity. There is evidence for a fucose-binding protein in boar spermatozoa (Topfer-Petersen et al. 1985) and maximum α-L-fucosidase activity was detected in porcine oviductal fluid close to the time of ovulation (Coy, unpublished observations). Moreover, treatment of the oviductal epithelium with fucosidase, or the presence of fucose prevented bull sperm binding to oviductal cells (Lefebvre et al. 1997; Suarez 1998; Ignotz et al. 2007). Consequently, the oviductal L-fucosidase could regulate the fucose residues present in the oviductal epithelium and control the releasing of the spermatozoa from the isthmus reservoir to reach the oocyte in the ampullar-isthmic junction. Because a galactose-binding protein has been identified in spermatozoa from equine (Dobrinski et al. 1996) and rat (Abdullah and Kierszenbaum 1989), it could be a possibility that oviductal β-D-galactosidase also participate in the release of the spermatozoa from the isthmus reservoir, as proposed for α-L-fucosidase. Similarly, β-N-acetyl-galactosaminidase and α-D-mannosidase activities in the porcine oviduct fluid reached their maximum at the early follicular phase (Coy et al. unpublished observations) and their corresponding sugar residues have been detected by lectin studies in porcine oviduct (Walter and Bavdek 1997; Sant'ana et al. 2005). Therefore, oviductal hexosaminidases and α-D-mannosidase might also have a role in remodelling the oviduct surface affecting sperm interaction with oviductal cells.

In addition to the chemical composition of the immediate surroundings of the spermatozoa, physical factors that influence the progress of sperm through the oviducts exist. A striking anatomical feature of the isthmus is the distinct layer of smooth muscle with cholinergic and adrenergic receptors (Brundin 1965; el-Banna and Hafez 1970; Hunter 1995, 1996). At least the latter are stimulated by oestrogens, which lead to a contraction of the smooth muscle (Hunter 1996). Furthermore, oestrogens increase the height of the epithelial cell layer (McDaniel et al. 1968) and induce oedema of the oviductal wall (Boyle et al. 1987). All single events together lead to a considerable obstruction of the oviducts, which might control the ascendance of

spermatozoa from isthmus to ampulla. It has also been hypothesized that the temperature gradient found to exist between isthmus and ampulla in the pre-ovulatory oviducts of sows (Hunter and Nichol 1986) and rabbit does (David et al. 1971) may be involved in governing sperm storage and capacitation. Alternatively, the gradient might only mirror differences in blood flow and smooth muscle activation between these two regions.

Interestingly, at least in pigs, the hormonal control over the management of the sperm reservoir seems to work not only via their indirect influence on environmental conditions but also the presence of oestrogens has been shown to increase the number of sperm binding to oviduct explants *in vitro* (Raychoudhury and Suarez 1991; Suarez et al. 1991). This indicates that a direct modulation of the binding properties is also possible. However, *in vitro* trials indicate that this is not the case in cattle (Lefebvre et al. 1995). Thus direct influence of gonadal steroids on sperm interactions seems not to be a concept common to all species.

Not only the regulation, but also the function and biological relevance of the sperm reservoir, is still not fully understood. Besides storage of competed spermatozoa and their timely release, it seems to be involved in preparing spermatozoa for fertilization. Bull sperm, for example, have been proven to have better oocyte penetration rates after co-culture with oviduct explants (Chian and Sirard 1995). A key factor appears to be the regulation of capacitation. Several studies have shown that sperm–OEC binding suppresses capacitation and thus prolongs the life-span of the attached spermatozoa (Smith and Yanagimachi 1991; Dobrinski et al. 1996; Smith and Nothnick 1997; Rodriguez-Martinez et al. 2001). On the other hand, as mentioned above, capacitational changes are thought to be responsible for the detachment of spermatozoa from the oviductal epithelium (Bosch and Wright 2005). Thus it has to be assumed that proximity of ovulation is somehow signalled to the oviducts, via hormonal changes or other, which in turn causes a switch in the oviduct environmental conditions from anti- to pro-capacitational. Whether the switch is communicated to the spermatozoa directly, via the binding site for instance, or by secretion of special capacitating factors into the oviduct lumen, or both, needs to be determined.

Another possible task for the oviduct sperm reservoir frequently mentioned in the literature is sperm selection. Evidence remains so far circumstantial, but nevertheless convincing. It has been noticed, for instance, that *in vitro,* only a subpopulation of all motile and morphologically intact spermatozoa actually binds to the epithelial cells (Thomas et al. 1994b). Furthermore, *in vivo* trials revealed that after insemination, intact spermatozoa were found anterior rather than posterior to the utero-tubal junction (Asch 1976; Mortimer et al. 1982). In addition, it has been demonstrated that epithelial-bound spermatozoa have a lower incidence of DNA-fragments in their chromatin (Ellington et al. 1999).

Finally, a novel path in the oviductal selection of spermatozoa has recently been described in mouse (Rodeheffer and Shur 2004), pig and cow (Coy et al., unpublished observations) and human (Munuce et al. 2008). From these studies, it seems clear that oviductal proteins modify the zona pellucida (ZP) of freshly ovulated oocytes thus affecting interaction with spermatozoa. While in mouse, the association of a 250 kDa, wheat germ agglutinin (WGA)-reactive described glycoprotein with the ZP facilitates sperm adhesion (Rodeheffer and Shur 2004), in human, pig and cattle, the findings are apparently the opposite. Incubation of human spermatozoa in the presence of proteins obtained from fallopian tubes reduced sperm affinity for the ZP, the effect being partially attributed to the decreased expression of D-mannose binding sites on the sperm surface (Munuce et al. 2008). In pig and cow, the oviduct-specific glycoprotein (OGP) has been identified as the responsible factor modifying the ZP resistance to proteolysis and consequently, affecting ZP-sperm binding, penetration and monospermy (Cánovas et al. 2008; Coy et al. 2008). The effect is not species specific and can be reverted by incubation of the oocytes in medium without heparin (Coy et al. 2008). The direct consequence of these findings is that either only spermatozoa capable to cross the reinforced physical barrier conformed by OGP-SGAGs and ZP (in pig and cow), or those carrying the specific ligand to bind the receptor on the oviduct-modified ZP (in human and mouse species), or both simultaneously, will successfully fertilize the oocyte. This clearly indicates that the oviducts select specific sperm subpopulations.

The above is to a certain extent already part of the final selection of spermatozoa that occurs during sperm–oocyte interactions. It involves three steps: first with cumulus cells and its hyaluronic acid extracellular matrix, second with the ZP and third with the oocyte plasma membrane. The primary interaction of the gametes is reversible and several sperm proteins are involved. Binding to ZP glycoproteins induces spermatozoa to undergo the acrosome reaction with the release of their contents. The acrosome reaction is a prerequisite for the further fusion process of the gametes. As capacitation is required for sperm to undergo acrosome reaction, only those spermatozoa with a functionally intact membrane system will be able to fertilize (Evans 2003). The secondary binding is irreversible as binding of zona glycoproteins and different specific sperm proteins occur. Interestingly, not only the carbohydrate structure itself but also its position in the molecule and the three dimensional structure of the ZP affect the binding functionability (Dunbar et al. 1994). A recent scanning electron microscopic study found quite different surface structures of the ZP depending on its maturation grade. The distribution pattern of spermatozoa on the zona was very variable and sperm penetration was shown not to be only an active process solely by the spermatozoa. The ZP as well, was actively involved in this process by overgrowing the sperm head with zona material (Rath et al. 2005).

Author contributions

All authors have contributed equally to this paper.

Conflicts of interest

The authors have declared no conflicts of interest.

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